

## **Evaluation of toxin producing snails with particular reference to *Conus* sp. at select locations along East Coast of India**

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### **Introduction:**

Conotoxins appear to have an important role in therapeutics and they are known for their specificity in blocking the ion-channel. Much of the work on conotoxins has been carried out on snails from Pacific Coast and there are only few reports on these snails from tropical coast. An attempt has been made to make a preliminary survey on *Conus* sp. along Visakhapatnam Coast and this resulted in identification of sixteen species. Among these snails, some are endemic in nature and therefore, addition exhaustive efforts have to be made to study their distribution at select locations on the east coast of India. The toxins from these snails will be isolated and characterized for their potency.

### **Objectives:**

- To collect and identify different species of *Conus* at select locations on the East Coast of India namely Visakhapatnam, Kakinada, Kalingapatnam and Paradeep.
- To study the histology and histochemistry of venom apparatus of *Conus* sp. and to identify the peptide toxins using immunocytochemical techniques
- To evaluate the potency of peptide toxins in different species of *Conus* and to further characterize them using MS and MALDI-TOF

### **Tasks to be undertaken:**

#### **Task 1: Collection and identification of *Conus***

A thorough investigation shall be undertaken to determine the occurrence of *Conus* species along Visakhapatnam, Kakinada, Kalingapatnam and Paradeep coasts. Samples will be collected both from intertidal and subtidal levels at the above select locations. Our earlier studies revealed about sixteen species of *Conus* along Visakhapatnam Coast and Kakinada Bay and two of them for the first time. Exhaustive studies shall be undertaken to determine the distribution of *Conus* snails along Visakhapatnam Coast and Kakinada Bay since they seem to harbour diverse species. Attempts will be made to extend these studies towards northeast coast off India i.e., from Kalingapatnam to Paradeep. A dredge will be used to collect the snails from depths at about 20 to 30 meters. Intertidal species will be picked up during low tides. A few of them will be fixed for identification and confirmation of the species. The distribution of these snails will also be monitored by using appropriate standard techniques keeping in view the endemic nature of some of these species. These surveys would be informative and lead to mapping of the availability of *Conus* species at the above select locations on the east coast of India.

#### **Task 2: Isolation of venom gland and extraction of crude toxin**

Live snails will be sacrificed and their venom glands isolated for extraction of toxins. Our earlier work on five species of *Conus* revealed differences in morphological and anatomical structure of the gland. The radular tooth of each of these species was also specialized to eject the toxin depending on its feeding behaviour. Therefore, the



morphological and anatomical details of the glands will be studied using standard histological and histochemical techniques. As these toxins are rich in S-S and -SH proteins, appropriate immunocytochemical techniques will be employed to identify specific small peptides. The morphological details of radular tooth will be studied in detail to understand the feeding behaviour of these snails so that a correlation can be made with the nature of the toxin and its prey.

### Task 3: Analysis and characterization of toxins

The crude venom from these snails will be extracted with 50% distilled water/acetonitrile and analyzed using RP-HPLC. Further characterization and fingerprinting of the peptides will be carried out by Mass Spectroscopy with the ongoing collaboration with Molecular Biology Unit of Indian Institute of Science. Earlier studies on five select species of *Conus* namely *C. figulinus*, *C. betulinus*, *C. loroisii*, *C. biliosus* and *C. inscriptus* revealed 20 putative peptides with one or two or three Cys-Cys disulphide linkages and their molecular weights ranged from 833 to 4386Da. The isolated peptides will be used to test their potency and those peptides showing novel activity will be studied using fish or mouse bioassay. Earlier studies with crude venom injected into fish showed changes in brain indicating neuro-degeneration. Some of the peptides are known for their activity in blocking specific receptors or channels. Therefore, suitable investigations will be undertaken to determine their pharmaceutical efficacy.

### Statement of the grant for the proposal (3 years)

| S.No. | Particulars   | Amount (Rs.)         |                      |                      |                  |
|-------|---|----------------------|----------------------|----------------------|------------------|
|       |   | 1 <sup>st</sup> Year | 2 <sup>nd</sup> Year | 3 <sup>rd</sup> Year | Total            |
| 1.    | Manpower<br>(Two Junior Research Fellows: @Rs.12,000pm for first two years and Rs.14,000pm for the third year plus 15% HRA)   | 3,31,200             | 3,31,200             | 3,86,400             | 10,48,800        |
|       | Lab/Technical Assts (4) & Deckhands (2) @ Rs.5,000pm  | 3,60,000             | 3,60,000             | 3,60,000             | 10,80,000        |
| 2.    | Contractual services: Professional services by AU Professors (Rtd.) (2) @ Rs.10,000 & Scientists of ZSI (Rtd.) (2) @ Rs.5,000 | 3,60,000             | 3,60,000             | 3,60,000             | 10,80,000        |
| 3.    | Boat hire and Travel (including trips to IISc)  | 4,00,000             | 4,00,000             | 2,00,000             | 10,00,000        |
| 4.    | Chemicals & Glassware   | 1,50,000             | 1,50,000             | 1,00,000             | 4,00,000         |
| 5.    | Contingency   | 1,00,000             | 1,00,000             | 1,00,000             | 3,00,000         |
| 6.    | Overheads   | 2,55,180             | 2,55,180             | 2,25,960             | 7,36,320         |
|       | <b>Total</b>  | <b>19,56,380</b>     | <b>19,56,380</b>     | <b>18,32,360</b>     | <b>56,45,120</b> |